RERF update RERF

News & Views from the US–Japan Radiation Effects Research Foundation

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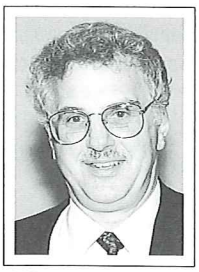
National Academy of Sciences President Visits RERF

On 11 November, US National Academy of Sciences (NAS) President **Bruce Alberts** visited the RERF Hiroshima Laboratory during a 2-day stay in the city.



Alberts

A biochemist renowned for his extensive study of protein complexes that allow chromosomes to replicate, Alberts met individually with research department chiefs in the morning to gain perspective on the day-to-day work ongoing at the laboratories in Hiroshima and Nagasaki.

Later in the day, he spoke before a packed auditorium about molecular "train wrecks" or colliding DNA polymerase and RNA polymerase. In less than an hour, he adeptly described years of research and the efforts of numerous collaborators who helped unravel the complexities and flexibilities of DNA transcription and replication.

A professor at the University of California–San Francisco, Alberts has long been involved in activities to improve science education in the US. He was elected a member of the Academy in 1981 and became president in 1993.

NAS Officials Call on Mayors of Hiroshima and Nagasaki

On 10 November, Alberts visited Hiroshima Peace Memorial Museum and reciprocated Hiroshima Mayor **Takashi Hiraoka**'s visit to NAS headquarters in Washington, DC, earlier in the year. Alberts presented to the mayor a certificate of gratitude and a medallion from NAS for the decades of cooperation extended to the ABCC–RERF research program by the citizens of Hiroshima.

On 26 January on behalf of Alberts who had been unable to visit Nagasaki in November, **John Zimbrick**, director of the NAS Board on Radiation Effects Research, presented to Nagasaki Mayor **Hitoshi Motoshima** the same tokens of appreciation.

Both mayors expressed favorable views of RERF's body of work. ❑

Removal of Mainframe Will Modernize RERF Computing

In January, RERF's rented mainframe computer was removed 15 months ahead of schedule. Now all laboratory computing in Hiroshima and Nagasaki is carried out using a combination of personal computers, Unix workstations, and the RERF communications network, according to Research Information Center Chief **Jill Ohara**.

"We wanted to replace the archaic computer architecture that was seriously limiting RERF's research and business activities," she explained. "Specifically, we wanted to improve data-management and analysis capabilities; to provide access to outside computing facilities, information databases, and communication networks already used by other research organizations; and to create an open-end system designed to more easily accommodate enhancements that researchers will need to remain competitive in the future. The subsequent budget crisis and funding uncertainty surrounding RERF during the last year or so increased the urgency of redirecting a portion of the mainframe rental funds to supplement RERF's restricted research budget." ❑

RERF Scientists Attend Chelyabinsk Meeting

Four RERF researchers attended the first international symposium on the effect of radiation accidents in the southern Ural Mountains of Russia, 10–12 January. More than 300 biophysicists and medical researchers from the US, Japan, Russia, Germany, and other countries participated in the 3-day meeting, sponsored by the Ural Research Center for Radiation Medicine, Chelyabinsk. Discussions centered on reevaluation of radiation doses and epidemiological follow-up of about 28,000 persons.

Participants representing RERF were **Mitoshi Akiyama**, chief, Department of Radiobiology; **Kiyohiko Mabuchi**, chief, Department of Epidemiology; **Nori Nakamura**, assistant chief, Department of Genetics; and **Dale Preston**, chief, Department of Statistics.

RERF scientists shared information on 1) the levels and patterns of excess solid-cancer and leukemia risk among the 87,000 Life Span Study atomic-bomb survivors, based upon 1950–1990 data; 2) the effects of several life-style risk factors on total mortality, as well as on cardiovascular mortality and cancer incidence among about 9500 men contacted first by mail survey in 1965; 3) a study of 1200 survivors to more clearly define the radiation-dose dependency of the somatic-cell mutant frequency; and 4) the use of electron-spin resonance of tooth enamel combined with measurements of chromosome aberration frequency to corroborate estimated doses. ❑

In This Issue

Perspectives

Informed Consent Procedures at RERF

by Donald Harkness*, RERF Update *Editor in Chief

Whereas patient welfare and safeguards to preserve the confidentiality of medical records have always been given high priority at ABCC–RERF, obtaining informed consent from study participants has only recently become a concern. This may not be too surprising since in the Japanese medical system patients are seldom given their diagnosis, prognosis, or options for therapy.

RERF established its Human Investigation Committee (HIC) in 1976, nearly 10 years after the US National Institutes of Health (NIH) had published its consensus guidelines for institutional review committees. No equivalent committee had existed during the days of ABCC.

The RERF HIC guidelines were revised in 1985 to include requirements to inform study subjects, preferably in writing, about the nature of all new research studies and to obtain their consent. Aside from an explanatory letter that was sent to members of the families being recruited for the genetics study (approved about the time of the guidelines revision) which involved preparation and storage of immortalized lymphocytes, these 1985 guidelines have been largely ignored. However, during the past year, in three instances RERF has made progress in its informed consent procedures.

In January 1994, a new research study to define more accurately the time of menopause in those exposed to radiation was initiated. The study requires that participants come to RERF every 6 months rather than every 2 years as in the Adult Health Study (AHS). There was great concern that study subjects would not cooperate with this more demanding schedule. A written script was prepared for use by the contactors when telephoning prospective participants, and a physicians' script was developed for further educating those who came in for the initial study visit. Patient cooperation for this study has turned out to be exceptionally high. Although consent in this case was given orally, written information was also used to "inform" the study subjects.

> *'. . . with respect to informed consent, RERF is well ahead of most medical research institutions in Japan.'*

One of the Institutional Review Boards at NIH, reviewing a US National Cancer Institute contract to the National Academy of Sciences in support of cancer-incidence studies at RERF, raised questions about our informed consent procedures for the AHS. Specifically the board members were concerned about our long-standing practice of storing plasma derived from residual blood

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News Briefs

✓ DOE–NAS Contract Extended through 30 September 1995

The US National Academy of Sciences (NAS) contract to coadminister the RERF research program with the Japanese Ministry of Health and Welfare has been extended through 30 September 1995, according to **John Zimbrick**, director of the NAS Board on Radiation Effects Research. US Department of Energy representatives report that during the 6-month NAS extension the agency intends to issue a request for proposal to solicit bids from other possible contractors.

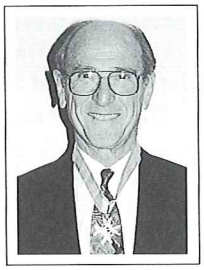
✓ Nagasaki Radiobiology Chief to Retire, Lab to Close

Department of Radiobiology Chief **Takeo Honda** will retire from RERF's Nagasaki Laboratory in March after 28 years of service.

As a result of Honda's retirement, the Laboratory of Cytogenetics in the Nagasaki Laboratory will close.

✓ RERF Vice Chairman Receives Imperial Award

On 24 November 1994, RERF Vice Chairman **Mortimer L Mendelsohn** was conferred with the Third Order of the Sacred Treasure—the highest imperial award bestowed upon those who are not citizens of Japan.



Mendelsohn

Cochairman of the RERF Scientific Council for 11 years, Mendelsohn was appointed an RERF permanent director in June 1992. He became vice chairman a year later. He was formerly associate director for biomedical and environmental research at Lawrence Livermore National Laboratory, where he worked for 20 years.

✓ Russian Academy Bestows RERF Chairman with Medal

For his distinguished service in the field of radiological medicine and radiation epidemiology, RERF Chairman **Itsuzo Shigematsu** has been awarded the Timofeev Medal by the Medical Radiological Research Center of the Russian Academy of Medical Sciences. The award commemorates N V Timofeev-Resovsky (1900–1981), a radiobiologist known for his neo-Darwinism and target theory.

Shigematsu chaired the International Atomic Energy Agency's post-Chernobyl environmental and public-health assessment project from 1990–91 and continues to forge cooperative research projects between RERF and researchers in the former Soviet Union who have been conducting long-term follow-up studies in radiation-contaminated areas.

✓ Historical Accounts of ABCC–RERF Published

Frank W Putnam, former RERF director (1982–87) and National Research Council Assembly of Life Sciences chairman (1977–1981), published a 30-page historical review article about ABCC–RERF in the journal *Perspectives in Biology and Medicine* (Vol 37, No. 4, pp 515–45, 1994). Reprints of this article can be obtained from the RERF Publication and Documentation Center.

In his autobiography *Physician to the Gene Pool—Genetic Lessons and Other*

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Two-dimensional Electrophoresis of End-labeled Genomic DNA Fragments

The author describes a highly efficient method that allows the simultaneous examination of more than 2000 DNA fragments for germinal mutations.

by Jun-ichi Asakawa, Laboratory of Biochemical Genetics, Department of Genetics, RERF

A principle focus of our research group is the study of spontaneous and induced germinal mutations caused by ionizing radiation in humans. We have been continuously searching for more efficient and more specific approaches than those of the past. Three years ago, I encountered an interesting technique, presented by I Hatada et al of the National Cardiovascular Center Research Institute at the 14th meeting of the Molecular Biology Society of Japan. I was greatly impressed by their new two-dimensional electrophoresis (2-DE) approach, which provided over 2000 DNA fragments (spots) from a genomic DNA digest without using any probes. This method, which they referred to as restriction landmark genome scanning, has utility in analyzing genomic alterations, including deletions and amplifications. To this end, we have refined this 2-DE technique as a mutation-screening method and have explored its potential use in our studies. To analyze the complex patterns of variation, we use computer algorithms developed for 2-DE protein gels by a research group of James V Neel and Samir M Hanash at the University of Michigan; computer analysis was carried out by computer scientist Rork Kuick. In this article, I will describe the present status of our efforts to identify in humans quantitative and qualitative variation in DNA spots visualized on autoradiographs of 2-DE sheet gels.

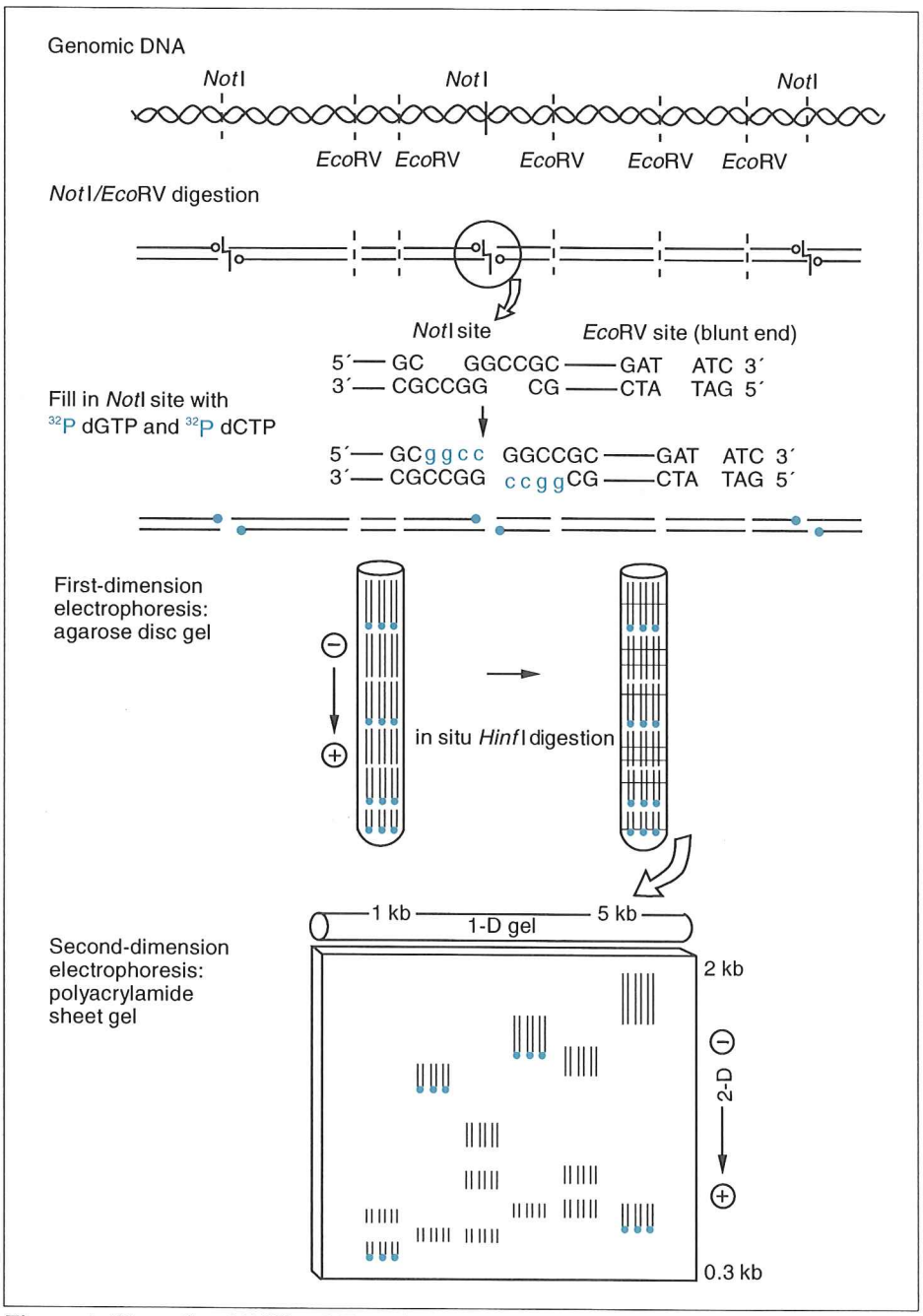

FIGURE BY K KANEOKA

Figure 1. The refined DNA 2-DE method. See 'Basic steps of 2-DE' below for a detailed explanation.

Basic steps of 2-DE

A genomic DNA is digested with restriction enzymes *Not*I and *Eco*RV (see Figure 1). The protruding ends produced by *Not*I are filled with ^{32}P-labeled deoxynucleotides by T7 polymerase (as shown in blue in Figure 1). This specific isotope-labeling of the *Not*I sites is the key distinc-

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Two-dimensional Electrophoresis

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tion of the 2-DE approach and results in specific visualization of DNA fragments containing the *Not*I site at one or both ends. The *Not*I sites are disproportionately frequent in the unmethylated "CpG islands" so common at the 5′ end of genes. Thus, the use of *Not*I as a restriction landmark enzyme may result in the derivation of a high proportion of the visualized fragments from active genes. The development of the current first-dimensional electrophoretic procedure has involved considerable experimentation. The RERF biochemical genetics program has used extensively in the past a disc gel electrophoretic system to study protein variants, and this system has been adapted for the DNA gels. After considerable experimentation, we obtained the best results with an agarose disc gel cast in Teflon tubing, with an inner diameter of 2.4 mm and 60 cm in length. I found that the uneven inner surface of the Teflon tubing stabilized the position of the agarose gel during electrophoresis. About 1 µg of DNA sample was electrophoresed. After electrophoresis for about 40 hours, the 32-cm portion of the disc gel containing DNA fragments approximately 1–5 kb in length was expelled from the tube and was incubated with restriction enzyme *Hinf*I. The *Hinf*I-digested DNA fragments were further separated perpendicularly on a polyacrylamide sheet gel (35 × 50 cm). The gels are dried and autoradiographed.

A digital image of the area of the autoradiogram analyzed is shown in Figure 2. On a single 2-DE DNA preparation made according to our conditions, approximately 2000 spots are visualized. The intensity of any spot that appears on the gel is usually expected to be determined by two homologous DNA fragments. In principle, this system will detect genetic variation of two types: 1) that due to gain or loss of a cut site for the three restriction enzymes employed in the study, and 2) that due to insertion/deletion/rearrangement (I/D/R) events. In the presence of a detectable variant in fragment length resulting from either 1) or 2) above, only one DNA fragment would be at the usual position, and the autoradiographic intensity of this spot should decrease by one-half. With respect to the variant fragment, it will either migrate to an altered position on the gel (a new spot), not enter the gel, or migrate off the gel. New spots may also appear on the gel as a result of variation in a fragment that does not normally appear on the gel. Some I/D/R events could eliminate a second fragment. With respect to either 1) or 2) above, homozygosity for the variant should be associated with the total disappearance of the corresponding normal spot. To enter into genetic analysis, these fragments must exhibit positional and quantitative stability.

For the quantitative studies of approximately 2000 spots on this image, 774 were selected as potential candidates because they were discrete, were not near the margins of the gel, and were not one of the large spots on the image. This analysis was limited to three mother/father/child trios run on two separate occasions. Of these 774 spots, 64 were singled out as "special" (the spot was judged to be a member of a genetic polymorphism) during preliminary matching. If spots that are the product of two homologous DNA fragments are to be distinguished with the requisite accu-

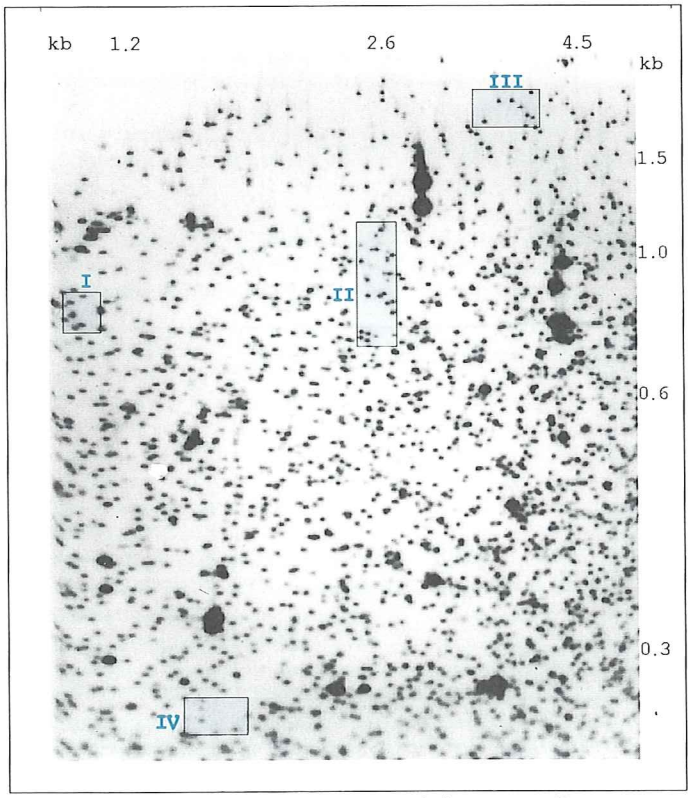

Figure 2. Digital image of the area of the autoradiogram analyzed. The fragment sizes in each dimension are indicated. Based on published DNA sequences, the position of most of the intense spots matches the predicted position of ribosomal DNA fragments or Epstein–Barr virus fragments. The variants pictured here in the numbered blue boxes correspond to the quadrants of Figure 3.

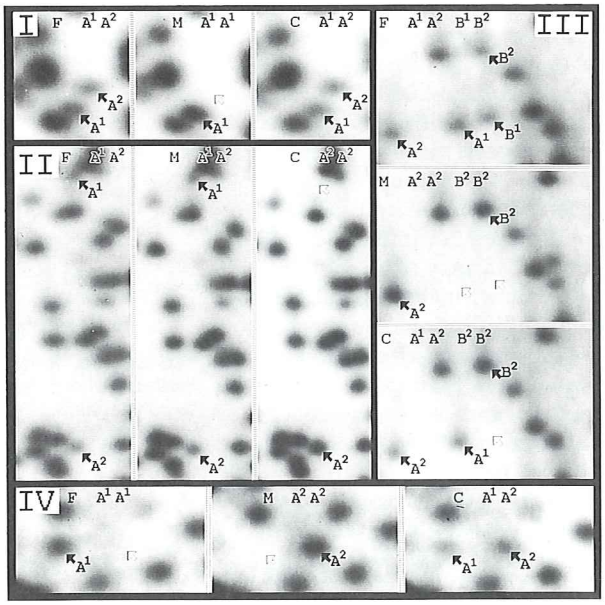

Figure 3. Types of polymorphisms observed. Panels I through IV each show sections of 2-D DNA gels for family trios. Individuals are labeled as follows: F = father, M = mother, and C = child. Arrows point to variable spots. Black arrows indicate spots present in the pattern, and white arrows point to the location of absent spots. The inferred genotypes for each person are indicated.

racy from spots that are the product of only one fragment, the coefficient of variation of spot intensity should be ≤ 0.12. At present, 439 of the spots in our preparations meet these standards. Particular attention will be directed at the current ability to distinguish between the autoradiographic intensity of a spot that is the product of two homologous DNA fragments as contrasted with the intensity of a fragment corresponding to one copy of the same DNA fragment. This is because in the usual case, a mutation will manifest itself as a spot of half-normal intensity in a child, both of whose parents have corresponding spots of normal intensity. This diminished spot intensity in the child may or may not be accompanied by the appearance of a new spot on the gel (Asakawa et al, *Proc Natl Acad Sci USA* 91:9052–6, 1994).

In the study of qualitative variation (ie, presence, absence, with no quantitative constraints), all of the autoradiograph was examined, including the 774 spots that were the basis for the quantitative studies. Besides the three family trios used in the quantitative study, three additional family trios were available for this study of qualitative variation, a total of six trios. In all, 392 fragments that varied in their occurrence among individuals were encountered. In no case did a fragment appear in a child's pattern without the same fragment being observed in at least one of the parents. Of these 392 spots, 184 could be organized into 85 polymorphic systems in which all alleles were detected. The 85 recognized polymorphic systems can be organized into four categories based on the positions of the spots representing the alleles, relative to each other. Examples of polymorphisms of each of these types are shown in Figure 3. This figure also illustrates that differences in relative spot intensity between heterozygotes (each one copy) and homozygotes (two-copy spots) are readily detectable. Figure 4 consists of diagrams of possible DNA variation leading to the location of the allelic fragments relative to each other on the gel for each type of polymorphism. Two additional fragments were always present in the patterns of fathers and the single male child in the study, but were absent in patterns of the five female children and of the mothers. In addition these spots were approximately half the intensity of most others in the pattern so that it is possible that these fragments are from the Y chromosome. The remaining 206 variable fragments were members of polymorphic systems in which all of the allelic fragments for the system were not identified. It is likely that many of these spots represent an allele of a polymorphic system for which another allele produces a fragment outside the size separation range of the gel. Alternatively, some of these spots could be related to each other as alleles of a single polymorphism, but the relatedness has not been established because of marked differences in the migration between allelic fragments.

FIGURE BY K KANEOKA

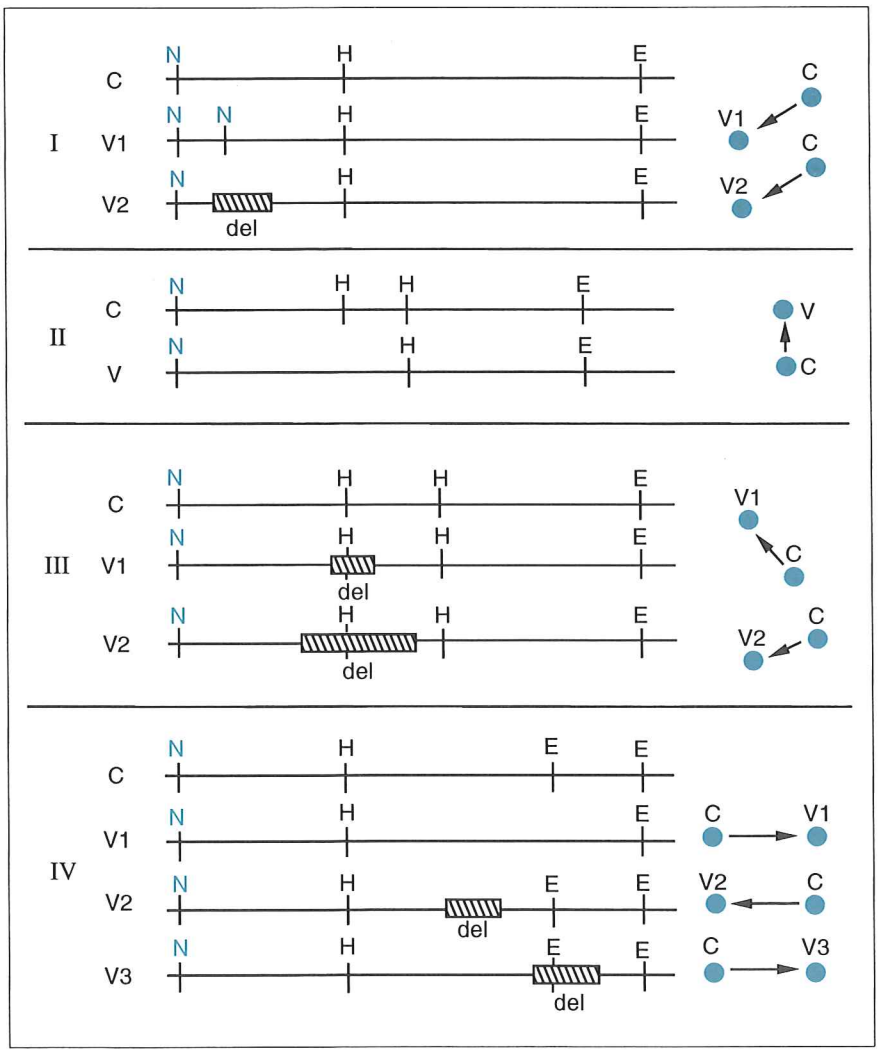

Figure 4. Restriction map diagrams showing possible reasons for the various spatial configurations of the allelic pairs of spots. Panels I through IV correspond with the panels of Figure 3 (see previous page). Fragments labeled C indicate the common allele with V1, V2, and V3 representing variants. Restriction sites are labeled as follows: N = NotI, H = HinfI, and E = EcoRV. Hatched rectangles labeled "del" represent deletions.

The 2-DE DNA separation system yielded remarkably reproducible 2-DE patterns. Numerous landmark invariant spots were present throughout the patterns for visual orientation, facilitating automatic spot matching and serving as size references for variable spots. The analysis of 2-DE patterns from six family trios revealed variability in the occurrence and intensity of a significant proportion of spots that appeared to be genetic in nature. The ability to visualize approximately 2000 fragments simultaneously, with variation in either separating dimension, undoubtedly increases the amount of information available relative to conventional 1-DE separations of genomic DNA fragments. These 2000 spots are derived from smaller, 1- to 5-kb, *Not*I–*Eco*RV fragments. We are now making an effort to develop a continuing 2-DE pattern for the bigger, 5- to 20-kb, *Not*I–*Eco*RV fragments that will visualize new 2000 spots. We believe that the power of this approach is augmented through the development of capabilities to characterize structural variants or mutants. Such studies, including development of a genomic DNA library, target cloning of DNA spots, and southern blotting of the 2-DE DNA gels are underway in collaboration with RERF research scientist M Kodaira. ❑

Site-specific Cancer Incidence: An Interim Report

Cancers that are thought to have some association with radiation exposure are being continually ascertained and pathologically confirmed in a series of RERF epidemiological studies.

by Shoji Tokuoka and Masayoshi Tokunaga, Department of Epidemiologic Pathology, RERF, and Kiyohiko Mabuchi, Department of Epidemiology, RERF

A series of pathological and epidemiological studies is in progress on various specific types of cancer in the RERF Life Span Study (LSS) cohort of atomic-bomb survivors and controls. Tumor cases are ascertained mainly from the Hiroshima and Nagasaki tumor and tissue registries, which include cases from university hospitals and other medical institutions in Hiroshima and Nagasaki, supplemented by clinical, autopsy, and surgical pathology records, tissue samples, and death-certificate information maintained at RERF. Case records and tissue samples are reviewed by a designated panel of two to four pathologists in Hiroshima and Nagasaki using internationally standardized diagnostic and classification criteria. The objectives are to confirm cancer cases by review of the pathology, as well as to ascertain cases from the time before the establishment of the Hiroshima and Nagasaki tumor and tissue registries.

Currently, study sites include the liver, salivary gland, skin, central nervous system (CNS), thyroid, ovary, breast, lymphoid tissue, and lung. The studies have been conducted under guidelines developed to provide uniformity in the study design and procedures (M Tokunaga et al, RERF Research Protocol 9-88). Analyses of the LSS cancer-incidence or mortality data have clearly indicated or suggested a relationship between tumors of these sites and radiation exposure. Case ascertainment and diagnostic confirmation have been completed for salivary gland tumor, skin cancer, CNS tumors, and liver cancer.

Salivary gland

JL Belsky et al previously indicated a significantly higher incidence ($p < 0.01$) of salivary-gland tumors among heavily exposed LSS groups, on the basis of 30 cases of salivary gland tumors (9 malignant and 21 benign) from 1957 to 1970 (*Cancer* 35:555–9, 1975). In the present study, a total of 145 cases of primary salivary-gland tumor was ascertained in the same LSS cohort from 1950 to 1987, among which 120 cases—more than 82%—were histologically reviewed by a panel of pathologists (T Saku, Y Hayashi, O Takahara, and H Matsuura).

The distribution of tumors by histological type showed a preponderance of mucoepidermoid carcinoma and adenoid cystic carcinoma among 35 malignant tumors—the two types accounting for 23 tumors (65.7%). Adenoid cystic carcinoma was the most common of the malignant tumors among both the nonexposed persons and those exposed to doses less than 1 Gy, followed by mucoepidermoid carcinoma. In the 1 Gy or higher group, not a single case of adenoid cystic carcinoma was found, whereas mucoepidermoid carcinoma accounted for 6 of the 8 malignant cases. A preliminary analysis revealed that the proportion of mucoepidermoid carcinomas among malignant tumors increased significantly with increasing radiation dose.

With 64 of 85 benign tumors having histological variation (75.3%), pleomorphic adenoma was the preponderant type of benign tumor, followed by adenolymphoma (synonymous with papillary cystadenoma lymphomatosum or Warthin's tumor) with 16 (18.8%) and 5 (5.9%) of the other types. In the 1 Gy or higher group, however, only 1 case of pleomorphic adenoma and 3 of adenolymphoma were observed. The proportion of adenolymphomas appeared to increase as the dose increased.

Skin

Recent studies suggest that the association between skin cancer and radiation exposure is considerably stronger than was previously thought. In the study of skin cancer among persons who underwent X-irradiation in childhood for treatment of tinea capitis, the excess relative risk of basal-cell carcinoma of the skin in the head and neck was 0.7 per gray and the average absolute risk was 0.31 per 10^4 person-year-gray (PYGy) (E Ron et al, *Radiat Res* 125:318–25, 1991). Recently, N Sadamori et al studied 47 cases of skin cancer that developed between 1958 and 1985 among LSS members in Nagasaki and found a highly significant dose-response relationship between A-bomb exposure and skin-cancer incidence (RERF Technical Report 10-91).

In the present study, a total of 343 skin-cancer cases was identified in Hiroshima and Nagasaki from 1950 to 1987. Of these, 259 cases were histologically confirmed by the pathologists (M Kishikawa, T Kobuke, and M Iseki): 231 single primary tumors and 28 multiple primary tumors. By location, cancers were relatively frequent on the face and legs. The histological distribution of all the cancer cases revealed that basal-cell carcinoma (36.8%) and squamous-cell carcinoma (34.6%) were the most frequent cancer types.

Preliminary analyses revealed a significantly elevated risk for basal-cell carcinoma, but no increased risk for squamous-cell carcinoma. Significant elevation of risk was also observed for Bowen's disease and other carcinomas. Among the youngest age group at the time of the bombings, a high excess relative risk for basal-cell carcinoma was remarkable.

Central nervous system

The brain and the spinal cord also seem to be relatively sensitive to the carcinogenic stimulus of radiation. Among Israelis who received childhood X-ray therapy for tinea capitis, an increased risk was evident not only for gliomas and meningiomas of the CNS, but also for nerve-sheath tumors of the peripheral nerve root. The frequency of these tumors was strongly related to radiation dose (E Ron et al, *N Engl J Med* 319:1033–9, 1988).

Analysis of site-specific cancer mortality in the LSS cohort conducted by Y Shimizu et al using DS86 doses

showed that the risk for brain tumors from 1950 to 1978 did not increase with DS86 dose (*Radiat Res* 121:120–41, 1990). Also, based on recent tumor-registry data, D Thompson et al reported that no dose response was observed for brain tumors; but for tumors of the nervous system except for the brain there was a suggestion of a dose response among those exposed when < 20 years old ($ERR_{1\ Sv}$ = 2.9, CI = –0.05–11.3). The frequency of meningioma was highest (43.2%), and the incidence rate was higher among women (*Radiat Res* 137:S17–S67, 1994).

In the present study, the ascertainment and pathology review of brain and other nervous-system tumors among the LSS population from 1950 to 1991 identified 374 tumor cases, in which 282 were histologically reviewed and diagnoses were confirmed by the pathologists (S Yonehara, H Fujii, M Kishikawa, and T Kobuke). Meningioma (41%) was most frequent, followed by schwannoma (or neurilemmoma) of the cranial and spinal nerves (25%), cerebral and spinal gliomas (19%), and anterior pituitary adenoma (11%).

Compared with CNS tumor incidence reported elsewhere, in the present study the proportional distribution of intracranial and spinal tumors by histological type showed a reverse relationship between the group of neuroepithelial tumors including glioma and the groups of meningeal as well as nerve-sheath tumors. The proportion of neuroepithelial tumors in the present case series was apparently small, and this tendency was unchanged even when the cases chosen for evaluation have pathology diagnoses based not on autopsy examination but on surgical pathology examination. Data analyses are currently underway.

Liver

The analysis of the recent tumor registry–based cancer-incidence data showed, for the first time in the LSS cohort, a significant association between liver cancer and radiation exposure. Previously, the prevalence of primary liver cancer among the LSS participants was found to be higher in Nagasaki than Hiroshima (WM Schreiber et al, ABCC Technical Report 15-69), and a subsequent study on the same cohort by M Asano et al reported 128 primary liver cancer cases from 1961 to 1975 (*J Natl Cancer Inst* 69:1221–7, 1982).

In the present study, the number of primary liver-cancer cases observed in the LSS population from 1958 to 1987 was 864 and, among these cases, 359—about 42%—were histologically reviewed with confirmation of the diagnoses by the pathology panel (T Fukuhara, H Itakura, and M Yamamoto). By histological type, these 359 cases included 303 hepatocellular carcinoma cases (84.4%) and 52 cholangiocarcinoma cases (14.5%). Of the hepatocellular carcinoma cases, 68% were accompanied by liver cirrhosis.

The present study also includes a nested case-control investigation of possible confounding or interactive effects of infection by the hepatitis virus. In parallel with the present studies, T Seyama and his colleagues at RERF are conducting molecular studies of hepatitis B and C viral infection (see T Mizuno et al, *RERF Update* 6[3]:6, 1994).

Thyroid gland

Among A-bomb survivors, thyroid disease has been a topic of extensive investigation. Besides prevalence studies of thyroid cancer among RERF's Adult Health Study participants, several incidence studies among the LSS population have been published. Most recently, S Akiba et al reported results from a study of 112 thyroid-cancer cases ascertained between 1958 and 1979 (RERF Technical Report 5-91).

In the present study, Y Hayashi and N Tsuda reviewed the histology of about 80% of cases for the period from 1950 to 1987. Thus far, about 428 malignant and about 231 benign tumor cases, totaling about 659 cases, have been confirmed as primary thyroid neoplasms.

Most of the malignant surgical cases (98.0%) were papillary carcinoma, and the majority of the papillary carcinoma cases were of the well-differentiated type. If papillary carcinoma can be defined as papillary microcarcinoma when its tissue specimens have a maximum focal diameter of 10 mm or less, then about 13% of all the papillary carcinoma cases could be classifed as such; however, 86% of papillary carcinoma cases diagnosed at autopsy were microcarcinoma. Interestingly, about 12% of the microcarcinoma cases in general were accompanied by the formation of metastatic foci in nearby lymph nodes. The large number of histologically reviewed cases in the present investigation will allow detailed analyses regarding issues that are important for risk estimation.

Other sites

Histological review is currently underway on ovarian tumors, breast cancer, lymphoid tumors, and lung cancer.

Site-specific cancer studies based on standardized pathology reviews, such as those described in this article, clearly will provide further pathological insights that might help us to better understand the nature, and possibly the mechanisms, of radiation risk. ❑

Perspectives

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drawn from AHS participants at each biennial examination. The participants had never been informed of this practice or that those samples had been, or might be in the future, used for unspecified research purposes. NIH rightfully took the position that AHS participants must be informed of this practice and give their consent. Because strong objections were raised over having AHS participants sign their names, a written consent form was designed on which "yes" or "no" could be designated. An RERF staff member then signs the form verifying the subject's choice. After a preliminary trial in which over 95% of persons asked gave their consent, the form was adopted in January 1995 for presentation to every member of the AHS cohort.

In the most recent example, a form was approved in late January 1995 on which a person may consent to the use for research of a small portion of the bone marrow removed at the time an aspiration procedure is being done for medical purposes. In this case, the study subject does sign the consent form and the physician who explains the study signs as a witness.

For the first time, RERF has adopted written consent documents through which study participants are able to designate their consent, either orally or by signature. Now that the inertia has been overcome and the HIC has become comfortable with these new practices, it should be reasonably easy to sustain them. Although RERF is only now adopting practices that have been the norm in the United States for 25 years, we find that with respect to informed consent, RERF is well ahead of most medical research institutions in Japan. ❑

In-utero-exposed Atomic-bomb Survivors: Cancer Risk Update

by Robert Delongchamp, Department of Statistics, and Yasuhiko Yoshimoto and Kiyohiko Mabuchi, Department of Epidemiology, RERF

Recently we updated the cancer incidence data for the in-utero mortality cohort with 5 more years of data, 1985–89 (Y Yoshimoto et al, *Lancet* 344:345–6, 1994). The accompanying tables summarize basic follow-up data from the cohort and show the effects of several changes made after the reporting of data through 1984 (Y Yoshimoto et al, *Lancet* 2:665–9, 1988). In addition to incidence data for 1985–89, some technical changes affect the 1950–1984 data. The first two columns in the tables show the data before and after these changes. The last column provides the new data.

The cohort size decreased from 1630 subjects to 1413 because those persons lacking Dosimetry System 1986 (DS86) doses were excluded. The earlier analysis had used ad hoc doses based upon an empirical adjustment of tentative 1965 dosimetry (T65D) doses for subjects without DS86 doses. Reducing the number of subjects caused a corresponding decrease in person-years and cases. The restriction to DS86 excluded two cancer cases. Another cancer case was excluded because the original diagnosis was revised after necropsy. However, the net change in cases is only one because two additional cancer cases diagnosed before 1984 were ascertained. In addition, some minor changes in doses occurred among the remaining subjects because the current dosimetry is a later version of DS86 (Version 3). Although the technical changes affect a large portion of the data, these changes do not alter prior conclusions. In particular, the dose effect during the 1950–1984 follow-up period is significant. To see this in Table 2, note that the 90% confidence limits for the excess relative risk have lower bounds that are greater than zero.

During the period 1985–89, seven cancer cases were recorded, a notable increase in cases relative to the previous period. However, these new cases do not reinforce the dose response. Our interpretation is that large relative risks applicable when this cohort was young have declined with advancing age. The point estimate for this period suggests that the excess risk has largely disappeared, whereas the upper 90% confidence bound cautions that substantial risks remain consistent with the data. Most of the expected cancer in this cohort will occur in future years since the subjects are only 49 years old. Hence, we caution against overinterpretation of the few cases from the most recent 5-year period. ❑

Table 1. Cancer incidence in a cohort exposed in utero to radiation from the atomic bombings of Hiroshima and Nagasaki.

Maternal uterine dose (Gy)	Follow-up period: Previous report 1950–1984	After dosimetry and case changes 1950–1984	After dosimetry and case changes 1985–89
		Cohort size	
	1630	1413	1348
		Person-years	
0	21,770	18,671	2,913
≥0.01	29,041	25,764	3,796
Total	50,811	44,434	6,709
		No. of cancer cases	
0	5	5	4
≥0.01	13	12	3
Total	18	17	7
		Crude rate/100,000 person-years	
0	23.0	26.8	137.3
≥0.01	44.8	46.6	79.0
Total	34.7	38.3	104.3

Table 2. Excess relative risk of cancer in a cohort exposed in utero to the atomic bombings.

	Follow-up period: Previous report 1950–1984	After dosimetry and case changes 1950–1984	After dosimetry and case changes 1985–89
Excess relative risk per gray	2.77	2.06	<0
90% confidence limits	(0.39, 9.99)	(0.10, 7.55)	(<0, 1.39)

News Briefs

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Stories (John Wiley & Sons, 1994; 457 pp), long-time RERF research collaborator and US National Academy of Sciences member **James V Neel** devoted parts of three chapters to atomic-bomb survivor genetics research. A member of the first scientific survey team to visit Hiroshima and Nagasaki on behalf of the US National Research Council in late 1946, Neel has played a continuing role in ABCC–RERF research during the past 5 decades.

✓ Research Staff News

Hiroshima

Department of Genetics: Research scientist **Yoshiaki Kodama** is now chief of the Laboratory of Cytogenetics.

Research Information Center: Information Systems Laboratory assistant chief **Scott Pohlman** and research scientist **Robert L Allen** resigned in December 1994 to return to the United States.

Nagasaki

Department of Radiobiology: Research scientist **Masahiro Itoh** has been transferred from the Laboratory of Cytogenetics, which was closed in March, to the Laboratory of Cell Biology. ❑

RERF Reports Published in Japanese, 1989–1992

Editor's note: Since 1989, only selected Japanese-language RERF reports have been available, either produced in-house or purchased from journal publishers. Following is a complete listing of these reports, 1989–1992. Henceforth, the availability of Japanese-language reports will be indicated in the Recent Scientific Publications section of this newsletter. TR = Technical Report Series, CR = Commentary and Review Series.

Allowing for random errors in radiation exposure estimates for the atomic bomb survivor data. DA Pierce, DO Stram, M Vaeth. **RERF TR 2-89.**

Smoking and serum proteins in atomic bomb survivors in Hiroshima. DO Stram, S Akiba, K Neriishi, RG Stevens, Y Hosoda. **RERF TR 3-89.**

Abdominal ultrasonographic screening of Adult Health Study participants. WJ Russell, Y Higashi, T Fukuya, Y Hosoda, J Murakami, A Mizushima, A Kawashima, S Murayama, T Ohuchida, F Mihara, M Takagi, S Fujita. **RERF TR 10-89.**

Incidence of thoracic vertebral fractures among Adult Health Study participants, Hiroshima and Nagasaki, 1958–86. S Fujiwara, S Mizuno, Y Ochi, H Sasaki, K Kodama, WJ Russell, Y Hosoda. **RERF TR 12-89.**

Congenital malformations, stillbirths, and early mortality among the children of atomic bomb survivors: A reanalysis. M Otake, WJ Schull, JV Neel. **RERF TR 13-89.**

Serum ferritin and stomach cancer risk among A-bomb survivors. S Akiba, K Neriishi, WJ Blot, M Kabuto, RG Stevens, H Kato, CE Land. **RERF TR 14-89.**

The observed relationship between the occurrence of acute radiation sickness and subsequent cancer mortality among A-bomb survivors in Hiroshima and Nagasaki. K Neriishi, DO Stram, M Vaeth, S Mizuno, S Akiba. **RERF TR 18-89.**

Variations with time and age of the excess cancer risk among A-bomb survivors. DA Pierce, M Vaeth, DL Preston. **RERF TR 21-89.**

Organ doses to examinees during photofluorography, fluoroscopy and computed tomography. K Kato, S Antoku, S Sawada, T Wada, WJ Russell. **RERF TR 2-90.**

Frequency of malignant tumors during the first two decades of life in the offspring (F_1) of atomic bomb survivors. Y Yoshimoto, JV Neel, WJ Schull, H Kato, M Soda, R Eto, K Mabuchi. **RERF TR 4-90.**

Radiosensitivity of skin fibroblasts from atomic bomb survivors with and without breast cancer. S Ban, RB Setlow, MA Bender, H Ezaki, T Hiraoka, M Yamane, M Nishiki, K Dohi, AA Awa, RC Miller, DM Parry, JJ Mulvihill, GW Beebe. **RERF TR 6-90.**

An investigation of random errors in the DS86 dosimetry using data on chromosome aberrations and severe epilation. R Sposto, DO Stram, AA Awa. **RERF TR 7-90.**

Hyperparathyroidism among atomic bomb survivors in Hiroshima, 1986–88. S Fujiwara, H Ezaki, R Sposto, S Akiba, K Neriishi, K Kodama, K Yoshimitsu, Y Hosoda, K Shimaoka. **RERF TR 8-90.**

Mortality among the offspring (F_1) of atomic bomb survivors, 1946–85. Y Yoshimoto, WJ Schull, H Kato, JV Neel. **RERF TR 1-91.**

Life Span Study Report 11. Part 3. Noncancer mortality, 1950–85, based on the revised doses (DS86). Y Shimizu, H Kato, WJ Schull, DG Hoel. **RERF TR 2-91.**

The effect of diagnostic misclassification on noncancer and cancer mortality dose response in the RERF Life Span Study. R Sposto, DL Preston, Y Shimizu, K Mabuchi. **RERF TR 4-91.**

Thyroid cancer incidence among atomic bomb survivors, 1958–79. S Akiba, J Lubin, H Ezaki, E Ron, T Ishimaru, M Asano, Y Shimizu, H Kato. **RERF TR 5-91.**

Radon concentrations in residential housing in Hiroshima and Nagasaki. T Aoyama, EP Radford, H Yonehara, H Kato, M Sakanoue. **RERF TR 8-91.**

Differential effects of atomic bomb irradiation in inducing major leukemia types: Analysis of open-city cases including the Life Span Study cohort based upon updated diagnostic systems and the dosimetry system 1986 (DS86). M Tomonaga, T Matsuo, RL Carter, JM Bennett, K Kuriyama, F Imanaka, S Kusumi, K Mabuchi, A Kuramoto, N Kamada, M Ichimaru, AV Pisciotta, SC Finch. **RERF TR 9-91.**

A longitudinal study of the association between ABO blood phenotype and total serum cholesterol level in the Adult Health Study, 1958–86. FL Wong, K Kodama, H Sasaki, M Yamada, HB Hamilton. **RERF TR 14-91.**

Radiation-related ophthalmologic changes and aging among the atomic bomb survivors: A reanalysis. M Otake, SC Finch, K Choshi, I Takaku, H Mishima, T Takase. **RERF TR 18-91.**

Cancer incidence in atomic bomb survivors. Part I. Use of the tumor registries in Hiroshima and Nagasaki for incidence studies. K Mabuchi, M Soda, E Ron, M Tokunaga, S Ochikubo, S Sugimoto, T Ikeda, M Terasaki, DL Preston, DE Thompson. **RERF CR 3-91.**

Adult Health Study Report 7. Noncancer disease incidence in the atomic-bomb survivors, 1958–1986 (examination cycles 1–14). FL Wong, M Yamada, H Sasaki, K Kodama, S Akiba, K Shimaoka, Y Hosoda. **RERF TR 1-92.**

Monoclonal gammopathy in atomic-bomb survivors. K Neriishi, Y Yoshimoto, RL Carter, T Matsuo, M Ichimaru, M Mikami, T Abe, K Fujimura, A Kuramoto. **RERF TR 2-92.**

Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958–1987. DE Thompson, K Mabuchi, E Ron, M Soda, M Tokunaga, S Ochikubo, S Sugimoto, T Ikeda, M Terasaki, S Izumi, DL Preston. **RERF TR 5-92.**

Radiation-related small head sizes among prenatally exposed atomic bomb survivors. M Otake, WJ Schull. **RERF TR 6-92.**

Proliferative and nonproliferative breast disease in atomic-bomb survivors: Results of a histopathology review of autopsy breast tissue. M Tokunaga, CE Land, Y Aoki, T Yamamoto, M Asano, E Sato, S Tokuoka, G Sakamoto, DL Page. **RERF TR 9-92.**

Radiation cataracts among Hiroshima atomic-bomb survivors, 1949–64. WJ Schull, M Otake, S Funamoto. **RERF TR 11-92.**

Thyroid diseases among atomic bomb survivors in Nagasaki. S Nagataki, Y Shibata, S Inoue, N Yokoyama, M Izumi, K Shimaoka. **RERF TR 12-92.** Published in *JAMA* (Japan) 15(12):64–73, 1994.

Colorectal cancer incidence among atomic bomb survivors, 1950–80. H Nakatsuka, Y Shimizu, T Yamamoto, I Sekine, H Ezaki, E Tahara, M Takahashi, T Shimoyama, N Mochinaga, M Tomita, R Tsuchiya, CE Land. **RERF TR 15-92.**

Levels of parathyroid hormone and calcitonin in serum among atomic bomb survivors. S Fujiwara, R Sposto, M Shiraki, N Yokoyama, H Sasaki, K Kodama, K Shimaoka. **RERF TR 18-92.**

A longitudinal study of growth and development among prenatally exposed atomic-bomb survivors. M Otake, Y Fujikoshi, WJ Schull, S Izumi. **RERF TR 19-92.**

Cancer incidence in atomic bomb survivors. Part III: Leukemia, lymphoma, and multiple myeloma, 1950–1987. DL Preston, S Kusumi, M Tomonaga, S Izumi, E Ron, A Kuramoto, N Kamada, H Dohy, T Matsuo, H Nonaka, DE Thompson, M Soda, K Mabuchi. **RERF TR 24-92.** ❑

Recent Scientific Publications

Editor's note: In this section of RERF Update, *selected summaries of journal articles based on approved RERF manuscripts accompany the complete journal-article citations. Other summaries from selected journal articles published by RERF researchers may also be included here occasionally. Variation in title or text styles reflects different journal styles.* J *after the citation means a Japanese version will be available. Reprints, when available, can be obtained from the RERF Publication and Documentation Center, 5-2 Hijiyama Park, Minami-ku, Hiroshima, 732 Japan. Facsimile: 81-82-263-7279. Internet address: maruyama@rerf.or.jp*

Approved Research Protocols

Studies on lung-cancer incidence among the atomic-bomb survivors, 1950–90. H Egawa, T Matsuo, S Yonehara, Y Fujita, E Nakashima, M Soda, M Tokunaga, S Tokuoka, S Akiba, K Mabuchi, DL Preston, CE Land. **RERF RP 1-94.** J

The proposed study will evaluate lung-cancer incidence in the RERF Extended Life Span Study sample during the period 1950–90. Tumors will be ascertained from autopsy and surgical pathology records and death certificates maintained by RERF, as well as from the tumor and tissue registries and other major medical institutions in Hiroshima and Nagasaki. Analyses regarding the anatomical and histological distribution of tumors, as well as the shape of the dose-response curve, age at exposure, attained age, sex, and tobacco-smoking effects will be conducted.

Molecular analysis of hepatocellular carcinoma among atomic-bomb survivors. KS Iwamoto, T Seyama, T Mizuno, T Ito, N Nakamura, M Akiyama, M Tokunaga, S Tokuoka, T Fukuhara, M Yamamoto, H Itakura, T Ikeda, Y Fujita, K Mabuchi. **RERF RP 2-94.** J

Following a relatively long latency period, primary liver cancer has been seen more often among the atomic-bomb (A-bomb) survivors as compared with the general Japanese population. Additionally, HBs-antigen positivity occurs often among high-dose A-bomb survivors, and it is suspected that the HB and HC viruses have a major role in the development of hepatocellular carcinoma (HCC). The mechanisms of these observations have not been examined closely; moreover, the interactions of these etiological factors in the development of HCC is not known. However, hepatocarcinogenesis is believed to involve multiple stages that include the mutations of more than one tumor-suppressor gene. Because p53 dysfunction correlates with hepatitis viral infection and because ionizing radiation produces deletion mutations, which is sufficient to disable tumor-suppressor genes, study of these genes in parallel with the hepatitis B and especially C viruses may clarify some aspects of hepatocarcinogenesis.

Incidence of lymphoid malignancies among the atomic-bomb survivors, 1950–1990. K Nanba, T Matsuo, M Tokunaga, J Jubashi, Y Fujita, M Soda, H Dohy, N Kamada, M Tomonaga, LB Travis, DL Preston, K Mabuchi, CE Land, S Tokuoka. **RERF RP 3-94.** J

In this study, we will investigate the incidence of lymphoid malignancies, including malignant lymphoma (ML), multiple myeloma, and lymphoid leukemia between 1950 and 1990 in the RERF extended Life Span Study (LSS) population, according to the RERF guidelines for site-specific cancer incidence studies. The purpose of the proposed study is to characterize the LSS sample in terms of risk for the complete spectrum of lymphoid malignancies, by cell type, in relation to radiation dose from the atomic bombings and other factors. Emphasis will be placed on confirmation and classification of cases by standardized pathology review based on extensive case ascertainment from all sources of diagnostic information, including the tumor and tissue registries in Hiroshima and Nagasaki, death certificates, and autopsy and surgical records kept at RERF and outside institutions. On the basis of pathology review, ML cases will be classified as Hodgkin's disease or non-Hodgkin's lymphoma (NHL) and will be subclassified according to the Rye classification and modified working formulation scheme. Cases of NHL will also be subclassified by immunohistochemical studies into T-cell lymphomas, B-cell lymphomas, or others. Diagnosis of adult T-cell leukemia/lymphoma will be based on detection of proviral DNA of HTLV-I using polymerase chain reaction and other current technology. The study will also make full use of hematological, clinical, and other diagnostic information obtained by other investigators and archived by the leukemia registry.

Publications in the Open Literature

Radiation-associated lung cancer: a comparison of the histology of lung cancers in uranium miners and survivors of the atomic bombings of Hiroshima and Nagasaki. CE Land, Y Shimosato, G Saccomanno, S Tokuoka, O Auerbach, R Tateishi, SD Greenberg, S Nambu, D Carter, S Akiba, R Keehn, P Madigan, TJ Mason, M Tokunaga. *Radiat Res* 134: 234–43, 1993.

A binational panel of Japanese and American pulmonary pathologists reviewed tissue slides of lung cancer cases diagnosed among Japanese A-bomb survivors and American uranium miners and classified the cases according to histological subtype. Blind reviews were completed on slides from 92 uranium miners and 108 A-bomb survivors, without knowledge of population, sex, age, smoking history, or level of radiation exposure. Consensus diagnoses were obtained with respect to principal subtype, including squamous-cell cancer, small-cell cancer, adenocarcinoma, and less frequent subtypes. The results were analyzed in terms of population, radiation dose, and smoking history. As expected, the proportion of squamous-cell cancer was positively related to smoking history in both populations. The relative frequencies of small-cell cancer and adenocarcinoma were very different in the two populations, but this difference was accounted for adequately by differences in radiation dose or, more specifically, dose-based relative risk estimates based on published data. Radiation-induced cancers appeared more likely to be of the small-cell subtype, and less likely to be adenocarcinomas, in both populations. The data appeared to require no additional explanation in terms of radiation quality (α particles vs γ rays), uniform or local irradiation, inhaled vs external radiation source, or other population difference.

Reproducibility of major diagnoses in a binational study of lung cancer in uranium miners and atomic bomb survivors. R Keehn, O Auerbach, S Nambu, D Carter, Y Shimosato, SD Greenberg, R Tateishi, G Saccomanno, S Tokuoka, C Land. *Am J Clin Pathol* 101:478–82, 1994.

A binational panel of four Japanese and four American pathologists examined 208 pulmonary neoplasms, according to the World Health Organization (WHO) recommendations, second edition, for the histologic typing of lung tumors. The study design included independent evaluations by pathologists working alone, followed by group reviews. The individual evaluations, and their implications for reproducibility of the WHO recommendations, are reported. Consensus (agreement by six or more pathologists) with respect to major (ie, first digit) diagnosis was obtained for 76.4% of the cases. Consensus was ob-

Recent Scientific Publications

tained for 72.5% of the cases with any major diagnosis of small cell cancer; the comparable figures for adenocarcinoma and squamous cell carcinoma were 56% and 48%, respectively. American pathologists were twice as likely as Japanese pathologists to diagnose large-cell cancer, the only significant national difference. Consensus was far less frequent with the minor (ie, second digit) diagnosis categories. This study shows that lung cancers continue to be difficult to classify reproducibly.

Agreement between death certificate and autopsy diagnoses among atomic bomb survivors. E Ron, RL Carter, S Jablon, K Mabuchi. *Epidemiol* 5:48–56, 1994.

Based on the Atomic Bomb Casualty Commission/Radiation Effects Research Foundation series of over 5,000 autopsies, we examined death certificate accuracy for 12 disease categories and assessed the effect of potential modifying factors on agreement and accuracy. The overall percentage agreement between death certificate and autopsy diagnoses was only 52.5%. Although neoplasms had the highest detection rate, almost 25% of cancers diagnosed at autopsy were nevertheless missed on death certificates. Confirmation and detection rates were above 70% for neoplasms and external causes of death only. Confirmation rates were between 50 and 70% for infectious diseases and heart and other vascular diseases. Detection rates reached a similar level for infectious, cerebrovascular, and digestive diseases. Specificity rates were above 90% for all except the cerebrovascular disease category.

Overall agreement decreased with increasing age at death and was worse for deaths occurring outside of hospital. There was some suggestion that agreement improved over time, but no indication that radiation dose, sex, city of residence, or inclusion in a biennial clinical examination program influenced agreement. Since the inaccuracy of death certificate diagnoses can have major implications for health research and planning, it is important to be aware that their accuracy is low and that it can vary widely depending on cause, age, and place of death.

Comparison of numerical results of repeated measurements of height based on two growth curve models with random-effects and general covariance structures. M Otake, E Nakashima, Y Fujikoshi, RL Carter, S Tanaka, Y Kubo. *J Jpn Stat Soc* 24:1–14, 1994.

A numerical comparison of two growth curve models, one with a random-effects covariance structure, and the other a general covariance structure, was made for a complete data set of 455 individuals with measurements of stature conducted annually from ages 10 to 18 years. The components of the variance-covariance matrix of the estimators of regression coefficients for a random-effects covariance structure were larger than those of a general variance-covariance matrix, ranging from 1.1 to 3.0 for the ratios of the elements of diagonal matrices, and 2.4 for an off-diagonal matrix. The ratios of elements of two off-diagonal matrices were about 2.7 and 4.3 if evaluated by an absolute value of these ratios for all elements with opposite signs. While the absolute difference between components in the two cases are small, the test statistics are almost the same except for the test value of the sex difference. The results obtained from the two models show that both are valid for the interpretation of the data set. For the data used here, the general model seems to fit the annually measured data better than the random-effects model for females 10–18 years old at the time of examination (ATE). The fit between observed and expected values is better in the general model for males 16–18 years old ATE, but it is better in the random-effects model for males 10–15 years of age ATE. The Akaike Information Criterion (AIC) value for a complete data set of 455 individuals as a measure of goodness of fit was 20,953.76 for the random-effects covariance structure, while for the general covariance structure it was 19,013.40. The random-effects model permits the use of an incomplete data set for 1264 individuals with four or more measurements. However, the results obtained are almost equal to those of a complete data set.

Increased rate of spontaneous mitotic recombination in T lymphocytes from a Bloom's syndrome patient using a flow-cytometric assay at *HLA-A* locus. Y Kusunoki, T Hayashi, Y Hirai, J Kushiro, K Tatsumi, T Kurihara, M Zghal, MR Kamoun, H Takebe, A Jeffreys, N Nakamura, M Akiyama. *Jpn J Cancer Res* 85:610–8, 1994.

Bloom's syndrome (BS) is an autosomal recessive disorder conferring a high propensity for cancer and displaying a high degree of genetic instability; the frequency of sister chromatid exchange is characteristically 10 times above background. The symmetrical four-armed chromatid interchanges are much more readily detected in peripheral blood lymphocytes of BS patients, suggesting that the frequency of somatic recombination is also increased. In the present study, the rate of spontaneous loss of *HLA-A* allele expression was estimated following fluctuation analysis in cultured T lymphocytes using a flow-cytometric assay. It was found to be 10 times or more higher than normal in lymphocytes from a BS patient. Molecular and chromosome analyses showed that all 13 independent variants from the patient were most likely derived from somatic recombinations. Further tests for loss of heterozygosity at a closely linked proximal locus, *HLA-DQA1*, showed that as many as half of the recombinants retained heterozygosity irrespective of the donor. The results suggest that the HLA region is hyperrecombinogenic in somatic cells and that the elevated recombination rate in BS cells results from the general increase at ordinary sites and not from random creation of unusual sites for recombination.

Increased frequency of $CD4^-8^-$ T cells bearing T-cell receptor $\alpha\beta$ chains in peripheral blood of atomic bomb survivors exposed to high doses. Y Kusunoki, S Kyoizumi, Y Hirai, S Fujita, M Akiyama. *Radiat Res* 139:67–72, 1994.

A rare T-cell subpopulation, $CD4^-8^-$ $\alpha\beta$ T cells, may be differentiated through a pathway (or pathways) different from the pathway(s) of conventional $CD4^+$ or $CD8^+$ T cells. In the present study, the frequencies of $CD4^-8^-$ T cells in peripheral-blood $\alpha\beta$ T cells in 409 atomic bomb survivors (160 estimated to have been exposed to 1.5 Gy or more and 249 controls) were determined to investigate late effects of radiation on the composition of human T-cell subpopulations. The frequency of $CD4^-8^-$ $\alpha\beta$ T cells decreased significantly with the subject's age and was higher in females than males. A significant increase in the frequency was found in the survivors exposed to more than 1.5 Gy, suggesting that the previous radiation exposure altered differentiation and development of T cells.

Thyroid diseases among atomic bomb survivors in Nagasaki. S Nagataki, Y Shibata, S Inoue, N Yokoyama, M Izumi, K Shimaoka. *JAMA* 272:364–70, 1994. J = *JAMA* (Japan) 15(12):64–73, 1994.

To elucidate the current thyroid disease status for the Nagasaki Adult Health Study cohort, Radiation Effects Research Foundation, a survey study was conducted. Among cohort members of the Nagasaki Adult Health Study who received biennial health examinations from October 1984 to April 1987 (n = 2856), a total of 2587 subjects remained after exclusion of persons exposed in Hiroshima or in utero and those who were not in Nagasaki at the time of the bombing. Thyroid radiation dose by the dosimetry system established in 1986 was available for 1978 of the 2587 subjects. Thyroid diseases were diagnosed using uniform procedures including ultrasonic scanning. The relationship of the prevalence of each thyroid disease with

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thyroid radiation dose, sex, and age was analyzed using logistic models. A significant dose-response relationship was observed for solid nodules, which include cancer, adenoma, adenomatous goiter, and nodules without histological diagnosis, and for antibody-positive spontaneous hypothyroidism (autoimmune hypothyroidism) but not for other diseases. The prevalence of solid nodules showed a monotonic dose-response relationship, yet that of autoimmune hypothyroidism displayed a concave dose-response relationship reaching a maximum (±SE) level of 0.7 ± 0.2 Sv. The present study confirmed the results of previous studies by showing a significant increase in solid nodules with dose to the thyroid and demonstrated for the first time a significant increase in autoimmune disease among atomic bomb survivors. A concave dose-response relationship indicates the necessity for further studies on the effects of relatively low doses of radiation on thyroid disease.

Frequent involvement of visible chromosomal deletion in X-ray-induced mutants at the HLA-A locus in human T-lymphocytes. Y Kodama, J Kushiro, Y Hirai, Y Kusunoki, N Nakamura, M Akiyama, AA Awa. *Mutat Res* 309:63–72, 1994.

Mutant T-lymphocytes at the HLA-A locus were isolated using a recently developed flow-cytometric assay either immediately after drawing blood (in vivo mutants) or after X-irradiation in vitro. Mutants were subsequently propagated clonally for cytogenetic and molecular analyses. Among the 38 in vivo mutants, none contained an abnormal chromsome 6 on which the HLA-A locus resides (6p21.3). In contrast, mutants recovered after in vitro irradiation frequently carried abnormalities in the short arm of chromosome 6: 11/19 and 5/5 independent mutants for the 1-Gy and 2-Gy groups, respectively. Characteristically, the majority of the aberrations were deletions, commonly involving chromosome 6p21-p23. Because chromosomal deletions involving the selected gene are rare among radiation-induced mutants at the hypoxanthine phosphoribosyltransferase (chromosome X) and thymidine kinase (chromosome 17) loci, the HLA-A locus can be considered as highly prone to chromosomal deletions after radiation exposure. It is generally believed that ionizing radiation randomly breaks DNA, and the higher frequency of chromosomal deletions at the HLA-A locus is unlikely to be due to preferential induction but more likely to the better survivability of the deletion-bearing mutants. Consequently, the results suggest that the human genome is quite heterogeneous with regard to the survivability of cells bearing a chromosomal deletion including different loci.

A positive correlation between T-cell-receptor mutant frequencies and dicentric chromosome frequencies in lymphocytes from radiotherapy patients. KS Iwamoto, Y Hirai, S Umeki, Y Kusunoki, S Kyoizumi, T Kodama, K Ohama, N Nakamura, M Akiyama. *J Radiat Res* (Tokyo) 35:92–103, 1994.

Dose estimates for the assessment of future risks, following accidental exposure to radiation, for certain diseases such as cancer usually rely on both physical and biological quantitative analyses. A traditional biological method of choice is the measurement of chromosome aberration frequencies in peripheral-blood lymphocytes. However, thorough examination of large sample populations is time and labor intensive. Recently, it became possible to measure mutant frequencies in T lymphocytes; one method is a colony assay at the HPRT gene, and the other is a flow-cytometric assay at the T-cell-receptor (TCR) gene. To test for the possible use of these mutation assays, concurrent measurements were taken on blood samples from women who previously received a full course of radiation therapy for gynecological cancer. The results showed that the frequency of TCR mutants correlated reasonably well with that of dicentric chromosomes, whereas the frequency of HPRT mutants did not. Possible uses of the TCR mutation assay in combination with the conventional chromosome analysis or micronucleus assay after exposure of a relatively large population are discussed.

Activated RET oncogene in thyroid cancers of children from areas contaminated by Chernobyl accident. T Ito, T Seyama, KS Iwamoto, T Mizuno, ND Tronko, IV Komissarenko, ED Cherstovoy, Y Satow, N Takeichi, K Dohi, M Akiyama. *Lancet* 344:259, 1994 (letter to the editor).

Fallout radioactivity in soil and food samples in the Ukraine: measurements of iodine, plutonium, cesium, and strontium isotopes. M Hoshi, M Yamamoto, H Kawamura, K Shinohara, Y Shibata, MT Kozlenko, T Takatsuji, S Yamashita, H Namba, N Yokoyama, M Izumi, K Fujimura, VV Danilyuk, S Nagataki, A Kuramoto, S Okajima, K Kiikuni, I Shigematsu. *Health Phys* 67:187–91, 1994.

Evidence of radiation-induced reduction of height and body weight from repeated measurements of adults exposed in childhood to the atomic bombs. M Otake, Y Fujikoshi, S Funamoto, WJ Schull. *Radiat Res* 140:112–22, 1994.

Ultrasonographic abdominal screening of atomic bomb-exposed subjects. WJ Russell, Y Higashi, T Fukuya, Y Hosoda, J Murakami, A Kawashima, S Murayama, T Ohuchida, F Mihara, M Takagi, S Fujita. *Acta Radiol* 35:155–8, 1994.

Publication of Interest Using RERF Data

Information bias and lifetime mortality risks of radiation-induced cancer. Low LET radiation. LE Peterson, WJ Schull, BR Davis, PA Buffler. Washington, DC, US Nuclear Regulatory Commission, NUREG/GR-0011, 1994. 58 pp. ❑

RERF update RERF

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RERF conducts research and studies—for peaceful purposes—on the medical effects of radiation on humans with a view toward contributing to the maintenance of the health and welfare of atomic-bomb survivors and to the enhancement of the health of all mankind.

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Contributions to *Update* receive editorial review only and are not subjected to scientific peer review. Consequently, the opinions expressed herein are those of the authors only and do not necessarily reflect RERF policies or positions.

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